BIOLOGICAL PROCESSES IN THE DECOMPOSITION OF ORGANIC MATTER

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THE DECOMPOSITION of organic matter can be regarded as taking place through two fairly readily separable processes. The first is concerned with the decomposition of freshly added material in the form of litter and roots; this would correspond to Winogradsky's zymogenous stage. The second concerns the decomposition of the residual amorphous material often grouped under the term 'humus', which would correspond to Winogradsky's autochthonous stage.

The quantity of material decomposed is often considerable, and in any ecosystem which has achieved relative stability the annual amount approximately equals the annual increment from litter fall and root death (Ovington, 1962). Litter fall can vary from $1\frac{1}{2}$ tons per acre in temperate hardwoods to 20 tons or more per acre in tropical rain forests. The contribution from the root systems is much more difficult to estimate but is usually taken as approximately half the litter fall. The increments of litter in grassland conditions have not been so well estimated but appear to be of comparable orders of magnitude to those formed in forests. The net photosynthetic gain per acre seems to be relatively independent of the vegetation type, provided there is a closed vegetation.

A number of microbiological studies of the details associated with the decomposition of freshly added material have been carried out (Webster, 1956, 1957; Kendrick and Burges, 1962; Waid, 1957). In all the sequences studied so far, there is a substantial infection of the plant tissues prior to shedding and falling to the ground. The senescent leaves of *Pinus sylvestris* become heavily infected with a number of fungi which include both parasites and surface saprophytes. These cause an appreciable loss in dry weight of the organic material in the needle prior to needle fall. In the case of herbaceous plants such as nettles (*Urtica*), there is often a very extensive invasion of both leaf and stem tissue before it falls to the ground. The leaves and stems of grasses undergo considerable decomposition while still attached to the plant, and often it is not until months after the tissues have died that they fall and become incorporated in the surface litter.

Knowledge of the early stages of the decomposition of roots is still scanty. Many workers have shown that the outer cortex of young roots is invaded by a large number of species while the root is still functional and that it is very difficult to distinguish between some forms of symbiotic invasions, parasitic invasions and the entry of the early saprophytes (Garrett, 1956). The organisms which are associated with the invasion of tissues prior to shedding often form a fairly well marked group of species which are associated with such habitats. When the tissues are shed, there may be a phase in which these invaders undergo a burst of activity, often associated with spore production, which is followed by a gradual decrease in activity, and ultimately these early species disappear. Invasion by true litter or soil species begins as soon as the tissues are in contact with the ground. A wide range of potential colonizers appears to begin to attack the tissues, but very quickly there is a selection of species and only a relatively small number become established. Often there is a clear-cut distinction between species which are superficial, as is the case with *Sympodiella* on pine needles, and others which are deep-seated and attack the internal tissues, as does *Verticicladium* (Kendrick and Burges, 1962).

Table 1

Root surfaces	Outer cortex	Inner cortex
		(1) *Endophyte* ————————→ ?
		(2) Sterile dark hyphae———→
	(3) Sterile hyaline hyphae———→	
	(4) *Fusarium* spp.————————→	
(5) *Penicillium* spp.		
Zygomycetes, e.g.	*Mucor hiemalis* ⎫	
	Mortierella spp. ⎭ ——→	
(6) *Rhizoctonia* spp. ⎫		
Trichoderma viride ⎮		
Gliocladium roseum ⎬ ————————→		
Cladosporium herbarum ⎭		

During the decomposition of rye roots there is a similar well-marked localization and succession of fungi, as has been shown by Waid (1957). He has summarized the succession in rye grass roots (*Table 1*). While this phase of decay was active, the stele tissue appeared to be intact and possibly still functioning. Subsequent decomposition of the stele seems to be brought about by different microorganisms. Waid (1962) suggests that differences in oxygen tensions may play

an important part in causing the zonation of the fungi in the decomposing cortex.

Very little is known concerning the factors which determine which species become involved in the various stages of attack. For instance, in any particular soil there may be a wide range of fungi which are capable of decomposing cellulose, yet only one or two species will dominate any cellulose substrate. In one soil examined by Griffith and Jones (1963) there were about twenty species which, when tested, were shown to be able to decompose cellulose, yet when cellulose was added to the soil, only one or two of these appeared to take an active part in the decomposition. Altering pH or nutrient status altered the dominant cellulose decomposer. In one experiment, for example, the dominant cellulytic form in the natural soil was *Rhizophlyctis*; addition of phosphate led to dominance by *Streptomyces*, while nitrogen amendments changed the conditions and favoured *Penicillium*.

In the successions so far described in detail the initial attack is almost always microbiological. In acid conditions, fungi predominate but where the pH is above about 5·5, bacteria play a much more important part.

In soils with a relatively high base status and abundant surface moisture, bacteria are often the most abundant early colonizers. This colonization, however, seems to depend on the presence of a free film of water. There is not yet sufficient evidence to make any broad generalization of the relative importance of microbial and animal activity in the early stages of decomposition. Although it has been known for a long time that earthworms and small animals play an important part in comminution and burial of leaf material, there have been no precise investigations of the microbial activity associated with the process. Work is now in progress both in Holland and in England in an attempt to fill this gap in our knowledge.

The degree of mineralization carried out by the primary microbiological invaders varies considerably. In the decomposition of pine needles the primary waves of fungi are associated with the loss in dry weight of approximately 20 per cent of the needle. In natural grasslands a greater loss in dry weight can occur before the leaves and stems fall.

It seems that, after the primary microbiological phase, the process slows up and might well come to a standstill if it were not followed by animal activity. In pine litter (Kendrick and Burges, 1962) the action of mites seems to be very important in the succession. Following the initial sporulation of the fungi *Sympodiella*, *Helicoma* and *Verticicladium*, the mites, and to a lesser extent the collembola, remove all the surface hyphae and most of the spores. Endophagous species

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invade the inside of the needle, reducing much of it to a loose mass of faecal pellets derived partly from cell tissue and partly from hyphae. In the decomposition of dung, both under laboratory conditions and in the field, the initial fungal activity fades away after a few weeks, and unless there is extensive action by the larvae of a large number of flies and other insects, decomposition slows down virtually to a stand-still. Again, in studies carried out on the decomposition of grass litter, the evidence would suggest that the initial stages are micro-biological, but these decline and unless there is animal activity, either in the form of earthworms or other soil animals, the litter accumulates in a stagnant condition. This has been particularly well shown by van Rhee (1963) in his studies in Holland in association with the reclamation of the polders.

During the initial stages, the biochemical evidence suggests that the readily fermentable materials, such as starch, hemicellulose and pectins, disappear and that the protein is also attacked. Substrates such as pine needles also contain considerable amounts of complex phenolic materials. Very little is known about the decomposition of these, but histological examination suggests that they disappear during the early microbiological phase. Many of the phenolic substances in plants occur as glycosides. The initial attack on these would probably be a hydrolysis leading to the release and utilization of the sugar moiety. The fate of the phenolic residue is not known. Handley (1954) has suggested that, in mor, the phenolic materials play an important part in tanning the protein and forming a pro-tective layer on the cellulose of the plant wall. It has also been suggested that the phenolic materials leach out of the fallen leaves and play an active part in the podzolization process (Bloomfield, 1957). It is, however, extremely difficult to demonstrate any free phenols in most litter on the surface of the ground (Coulson, Davies and Lewis, 1960). Our own work on pine litter has likewise failed to demon-strate free phenols. It is possible that any phenols released which were not associated in the tanning of protein, as suggested by Handley, are oxidatively polymerized by microorganisms during the early stages of humic acid formation. Frequently microscopic examination of the leaf litter shows that darkening of the cell walls and the occurrence of black deposits on the epidermis occurs prior to any noticeable decomposition of lignin; however, it is not possible to say whether this represents the early stage of humic acid or not.

After the wave of animal activity, a change occurs in the general microbiological population of pine litter. Dense wefts of fungal mycelium become noticeable, matting the needles together. A high proportion of the fungi concerned have clamp connections and are

therefore assumed to be *Basidiomycetes*. This phase is correlated with the disappearance of most of the cellulose and lignin. At the same time, animal activity also increases. The action of both the fungi and animals gradually reduces the fallen material to a more or less amorphous mass when viewed with the naked eye. Microscopic examination, however, shows that this apparently amorphous mass is made up primarily of faecal pellets and plant debris, usually consisting of only one or two cells.

It seems that the major factors concerned with the initiation of the *Basidiomycete* phase are the increased density of the litter and, associated with this, a very much moister condition. Work now in progress on litter under *Pinus nigra* shows that where a very much denser and more closely packed litter is produced, the mats of *Basidiomycete* mycelium occur nearer to the surface of the general litter layer.

During the decomposition of the needles there is a progressive loss of carbon dioxide which leads to a substantial narrowing of the carbon/nitrogen ratio. It is difficult to place the matter on a satisfactory quantitative basis, but the evidence would suggest that there is very little loss of nitrogen in the L and F_1 layers, the plant protein being converted into microbiological protein. At the base of the F_2 layer, de-amination occurs and nitrogen is released as ammonia. We have been unable to find any evidence at all of nitrate formation. In view of the low pH (4·2) one would not expect bacterial nitrification, but the possibility exists that heterotrophic nitrate formation by fungi might occur in these soils; however, we have been unable to demonstrate this.

The H horizon is one very rich in residual nitrogenous material formed from the disintegrated faecal pellets, remains of fungal hyphae, exoskeletons of animals and particles of cuticle and suberized cell walls. It is a substrate particularly rich in chitin. Examination of this layer by Gray and Bell (1963) showed that there were a number of fungi, of which *Mortierellas* were particularly prominent, which occurred abundantly in the H horizon and which, under laboratory conditions, were capable of active decomposition of chitin.

As yet there have been no studies concerned with the decomposition of either suberin or cutin. One of the characteristic features of pine litter is the very heavy fall of pine pollen when the male cones open. These pollen grains are decomposed very rapidly, the wings disappearing in a few weeks and the remainder of the grain within one to two months, so that despite the tremendous fall of pollen it is difficult to detect pollen grains in the litter layers except for a brief period of the year.

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The final disintegration of the organic matter seems to be associated with the complete dispersion of the faecal pellets and the downward movement of the highly dispersed humic acid materials.

Because of the large amount of humic material which accumulates in soil, many workers have assumed that it must have come from one of the major fractions in the decomposing debris. There is no necessity for this to be so. When rates of humus accumulation are compared with the total litter fall, it is readily seen that it would be possible for all the humified organic matter to have come from materials which would account for only a fraction of a per cent of the original plant debris.

Studies on the Delamere site (Kendrick, 1959) have shown that it takes of the order of 8 to 10 years for the complete disintegration of a pine needle. At the other end of the time scale, the decomposition of a soft fern leaf in a tropical rain forest is usually only a matter of days or of one or two weeks.

The decomposition processes described above for pine litter are characteristic of those in which the base status of the litter and the general overall pH levels are such that bacterial action and, to a lesser extent, actinomycete action are at a very low level. The process is primarily a fungal–mite-controlled process, although collembola, millipedes and enchytraeids are also important. In many other situations a much wider range of animals is found. In the tropics, ants and termites are responsible for the comminution and burial of a major fraction of the plant debris. Birds and burrowing mammals are active in turning over the litter, in breaking it into smaller fragments and in incorporating it with the surface soil. The larger worms will transport plant debris from the surface to the lower layers of the soil, either by direct transport or after ingestion. There has, however, been little coordinated work in attempting to follow the succession of all the different biological groups involved in such decompositions.

In the mineral soil the distribution of organic material is found to be highly complex. In litter, and particularly that of pine, there is a simple spatial relationship, with the youngest stages of the succession at the top and the oldest at the bottom. A sampling sequence downwards through the litter gives what is now fashionably called a chronosequence. In the soil no such sequence exists: roots in the first stages of decay exist alongside the most resistant residues which represent the final end product of the decomposition. Theoretically it is possible readily to dissociate the decomposing roots from the other soil organic matter and to follow the successive stages in the way that leaf decomposition has been studied. While this can be done for

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the initial steps, it soon becomes increasingly difficult to detach and examine the crumbling root fragments. However, a consideration of such data as are available would suggest that, in general, root decomposition follows a similar path to leaf decomposition, but very different species are involved.

The overall rate of decomposition in the soil is much lower than in the litter if the measure is based on oxygen uptake per gramme of litter or soil, as shown in *Table 2*, (1). If, however, the results are expressed in relation to the amount of organic matter (2), it is seen that the rates of decomposition in the soil are comparable with some of the litter layers.

Table 2

Oxygen uptake by different podzol horizons

(From Parkinson and Coups, 1963)

	μl. O_2/5 h		
Horizon	(1) per g *dry soil*	(2) per g *organic matter*	(3) per cent *organic matter*
A L	2366	2406	98
F_1	1400	1428	98
F_2	245	274	89
H	81	148	54
A_1	13·3	77·7	17
A_2	4·5	238·8	1·8
B_1	9·8	91·9	10·6
B_2	2·9	56·6	5·2
C	1·4	96·3	1·4

There is reason to believe that a large proportion of this oxygen uptake in the mineral soil comes from the decomposition of roots and that resistant humic material contributes little. The contrast between the relative activity of the organic matter in the A_2 and in the B_1 horizons shown in *Table 2* is particularly interesting.

Knowledge of the chemical processes involved in the decomposition of the more resistant fractions of the soil organic material is still scanty. Interest has centred particularly on the humic fraction. Evidence is now fairly conclusive that humic acids are highly condensed phenolic polymers existing in a poly-dispersed state (Burges, 1960). Degradations of humic acids yield a variety of phenols which appear to be derived from several different sources (Burges *et al.*, 1963). Burges,

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Hurst and Walkden (1964) have suggested that phenols derived from flavonoid components of the fallen litter, together with those from lignin decomposition and perhaps also from microbial synthesis, are oxidatively polymerized. If free amino acids are present, they may be incorporated in the condensate. During the process carboxyl groups are formed. The nature of the phenols and their relative proportions will differ from place to place, as will the degree of condensation and the size of the ultimate polymer unit. If this is correct, then there is no such thing as a definable humic acid molecule; at best, we may be able to adopt ideas from clay chemistry and describe the different humic acids in terms of a phenolic framework, characterized by the ratios of different substitution patterns and the proportions of ether and carbon linkages forming the basic framework.

Microbiological work has shown that a wide range of fungi, mainly lignin decomposers, can decolourize humic acid under laboratory conditions. Decomposition is a highly aerobic process and is dependent on an accessory energy source. Despite all efforts, the author and his colleagues have so far failed completely to obtain any intermediate breakdown compounds. An examination of the fungi concerned has shown (Hurst, Burges and Latter, 1962) that those fungi which decompose humic acid are capable of reducing an aromatic carboxyl. One of the enzymes involved is a NADPH-demanding system.

The initial stages of decomposition of humic acid are associated with the adsorption of the acid onto the hyphae. Extraction of this acid and subsequent examination of the infra-red absorption spectrum led Hurst (1963) to believe that the first steps in decomposition of the humic acid were the reduction of the surface carboxyl groups.

If this is the mechanism, then one could postulate that the following situations could arise. The humic acid being oxidatively polymerized would be very stable and normally survive for long periods. Even if reducing conditions were produced in the soil, the acid would still be stable. For decomposition to occur there would have be an accessory carbon source and good aeration to allow the microorganisms to produce a supply of NADPH for the first step. Draining peaty soils would allow fungi to use residual carbohydrates, etc. and, with aeration, to produce the initial reducing power to break the humic molecule. The rapid wasting of organic soils, including the humic fraction, is thus explicable. In most well aerated soils the supply of readily usable carbohydrates is small, particularly in those situations where humic acids accumulate. The absence of abundant accessory energy would mean that the majority of the humic acid remained unattacked. Addition of fresh organic matter leads to rapid carbon

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dioxide evolution. Experiments in which labelled carbon substrates have been added to soil have shown an increase in carbon dioxide from non-labelled substrates. If the microorganisms were using the new carbon sources to obtain the NADPH to break the old carbon compounds in the way suggested above, this would explain some of the curious features of the well known priming effect of freshly added organic matter.

On first thoughts, it might seem strange that nature had ever evolved humic materials. It is, however, less difficult than it might seem to suggest how this had occurred. In soils with high ion exchange capacities associated with clay fractions, plants which induced humic acid formation would have no advantage. On the other hand, plants growing on sandy soils would suffer from the difficulties associated with a soil with negligible ion exchange capacity. Leaching losses would be serious for the plants; thus species which induced humic acid formation with its high cation exchange capacity would have a selective advantage. Anion exchange capacity would be much less important, particularly as under the typical mor conditions the nitrogen cycle is abbreviated; de-amination of proteins leads to the release of ammonia which can be readsorbed by the plants. It is interesting to note in this connection that humic acids have a much greater affinity for ammonia than the carboxyl groups alone would account for.

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